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(71) Applicant *(for all designated States except US):* MALLINCKRODT MEDICAL, INC. [US/US]; 675 McDonnell Blvd., P.O. Box 5840, St. Louis, MO 63134 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* VISSER, Theofilus, J. [NL/NL]; LAMBERTS, Steven, W., J. [NL/NL]; Octrooibureau Zoan B.V., C.J. van Houtenlaan 36, NL-Weesp (NL). KRENNING, Eric, P. [NL/NL]; Octrooibureau Zoan B.V., C.J. van Houtelaan 36, NL-Weesp (NL). BAKKER, Willem, H. [NL/NL]; VAN HAGEN, Petrus, M. [NL/NL]; Octrooibureau Zoan B.V., C.J. van Houtenlaan 36, NL-Weesp (NL).

(74) Agents: KLOSTERMANN, Roy, J. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

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(54) Title: METHOD FOR DETECTING AN LOCALIZING TISSUES HAVING NEUROKININE 1 RECEPTORS

(57) Abstract

The invention relates to a method for detecting and localizing tissues, having neurokinine 1 receptors, in the body of a warm-blooded living being, by administration of a composition comprising a labelled small peptide, having a selective affinity to neurokinine 1 receptors, and by then radioassaying said being. The invention also relates to a method for the therapeutic treatment of tumors, having on their surface neurokinine 1 receptors, in the body of a warm-blooded living being, by treating said being with a labelled small peptide, having selective affinity to neurokinine 1 receptors.

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METHOD FOR DETECTING AND LOCALIZING TISSUES HAVING NEUROKININE 1 RECEPTORS

The invention relates to a method for detecting and localizing tissues having neurokinine 1 receptors in the body of a warm-blooded living being. The invention also relates to the therapeutic treatment of tumors having on its surface neurokinine 1 receptors in the body of said being. The invention further relates to a pharmaceutical composition to be used for the above method and to a kit for preparing a radiopharmaceutical composition.

Binding studies performed in vitro have demonstrated that small peptides that participate in the regulation of diverse biological processes, as well as analogues thereof, have a high affinity to certain tissues. Neurokinine 1 (NK1) receptors are demonstrated in both the brain and peripheral tissues. Such tissues prefer interaction with certain small peptides such as substance P (SP) and related compounds. In these studies ^{125}I-Bolton Hunter derivatized analogues of SP and related compounds were used to determine the binding affinity of such small peptides to NK1 specific binding sites. A number of recent publications is devoted to these in vitro studies, wherein different tissue preparations of test animals are used, e.g. preparations of brain membranes, of synaptosomes, of duodenal membranes, of urinary bladders, of ilia, of carotid arteries and of salivary glands. Reference is made in this connection to the publications of Lavielle et al. in Biochem. Pharmacol. 37, 1988, 41-49; Regoli et al. in Pharmacology 38, 1989, 1-15; Lew et al. in Eur. J. Pharmacol. 184, 1990, 97-108; and Tousignant et al. in Brain Research 524, 1990, 263-270.

It is the object of the present invention to provide a method for detecting and localizing tissues having neurokinine 1 receptors in the body of a warm-blooded living being. Such a method would be a powerful tool in diagnosing various diseases and disorders, that are related to neurokinine 1 receptors in body tissues, in vivo, such as tumors with NK1 receptors, e.g. malignant glioma, pheochromocytoma, paraganglia and SCLC (small cell lung cancer), and in visualising NK1 receptors on certain tissues, such as regenerating nervous tissue, e.g. polyneuropathy, nervous section and other degenerative processes, in the central nervous system, including the spinal cord, and on tissue which shows an immunological reaction, e.g. in case of granuloma, lymphoma and Crohn's disease. In order to be able to achieve a specific therapy for the above diseases and disorders, the detection and localization of tissues having NK1

receptors in an early stage is of the utmost importance. In addition, a good diagnostic method is also indispensable for supporting the therapy used. Various requirements have to be imposed on an agent that is used in such a diagnostic method, for example, nontoxic, no adverse influence on the host resistance and/or the therapeutic treatment, well detectable and highly selective. The required high selectivity means that the diagnostic agent, after having been introduced into the body, must accumulate more strongly in the tissue or tissues to be detected or visualized than in surrounding tissues. This selectivity, i.e. a comparatively stronger concentration of the diagnostic agent in the target tissue or tissues compared with non-target tissues, enables the user to correctly diagnose the disease or disorder. In order to be detectable from outside the body, the diagnostic agent should be labelled, preferably with a radionuclide or with a paramagnetic metal isotope. In the former case, the radioactive radiation can be detected by using a suitable detector (scanning). Modern techniques in this field use emission tomography; when gamma radiating isotopes are used, the so-called single photon emission computerized tomography (SPECT) may be applied. The use of paramagnetic diagnostic agents enables a detection by means of imaging by magnetic resonance.

The above-defined object can be achieved according to the present invention by a method, which comprises (i) administering to said being a composition comprising, in a quantity sufficient for external imaging, a small peptide having a selective affinity to neurokinine 1 receptors, wherein said peptide is labelled with (a) a detectable metal isotope selected from the group consisting of Tc-99m, Pb-203, Ga-67, Ga-68, As-72, In-111, In-113m, Ru-97, Cu-62, Cu-64, Fe-52, Mn-52m, Cr-51, Na-23, Gd-157, Mn-55, Dy-162, Cr-52 and Fe-56, said metal isotope being attached to said peptide via a suitable linker capable of reacting with an amino group, preferably a terminal amino group, of said peptide, and having a chelating group for chelating said metal isotope, or with (b) a detectable halogen radioisotope selected from I-123, I-131, Br-75 and Br-76, said halogen radioisotope being attached to said peptide directly or via a tyrosyl linking group; and thereupon (ii) subjecting said being to external imaging to determine the targeted sites in the body of said being in relation to the background activity, in order to allow detection and localization of said tissues in said body.

The above labelled peptides, having a selective affinity to neurokinine 1 receptors, have been tested in a number of suitable model experiments that are

predictive for in vivo application. These experiments are described in the examples. From the results it will be evident, that the tested labelled peptides have properties which make them suitable as diagnostic agents. As will become apparent from the examples, the labelled peptide remains sufficiently long intact after administration to permit imaging of the target organ or tissue without a disturbing background activity, for example, due to detached label. Further it is of utmost importance, that the method of the present invention is also well suitable for detecting and localizing tissues having neurokinine 1 receptors, when these tissues are present in the abdominal region of the living being, e.g. for detecting and localizing certain tumors in the abdominal cavity and for visualizing Crohn's disease. As will become apparent from the examples, viz. the liver perfusion model experiments described therein, the tested labelled peptides of the present invention show such a slow liver metabolic clearance, that only a small background activity will result, not only in the abdominal region but also in the circulation system. These metabolic properties, as determined by the model experiments described above and illustrated in the Examples, make the labelled peptide particularly suitable for the method of the invention, because a favourable target tissue to background ratio may be expected. On the contrary, ^{125}I-Bolton Hunter modified SP, known from the above-mentioned in vitro binding studies, shows a relatively fast liver metabolic clearance, which makes this substance significantly less suitable for in vivo application; this will be clear from the appended Examples.

It is another object of the invention to provide a method for the therapeutic treatment of tumors having on their surface neurokinine 1 receptors in the body of a warm-blooded living being.

This object can be achieved according to a different aspect of the present invention by administering to said being a composition comprising, in a quantity effective for combating or controlling tumors, a small peptide as defined above, if said peptide has been labelled via a suitable linker, as mentioned above, with an isotope suitable for the purpose in view. Suitable isotopes for combating or controlling tumors are beta-emitting isotopes such as Re-186, Re-188, As-77, Y-90, Cu-67, Er-169, Sn-121, Te-127, Pr-142, Pr-143, Au-198, Pd-109 and Dy-165.

The selective affinity of the above labelled peptides to neurokinine 1 receptors make these labelled compounds particular suitable for therapeutic treatment of certain malignant tumors that are related to neurokinine 1 binding places, such as malignant glioma, pheochromocytoma, paraganglia and SCLC.

The labelled peptide to be used according to the method of the invention is preferably derived from a compound of the general formula

$$R_1\text{-}(A_1)_m\text{-}A_2)_n\text{-}A_3)_o\text{-}Pro)_p\text{-}A_4)_q\text{-}A_5\text{-}Phe\text{-}A_6\text{-}A_7\text{-}A_8\text{-}Met(O)_s\text{-}R_2 \quad (I)$$

wherein all of the symbols m, n, o, p and q are 1, or all but one of the symbols m, n, o, p and q are 1 and the remaining symbol is 0;
R_1 is a hydrogen atom or a C_1-C_4 alkylcarbonyl group;
R_2 is an amino group, a hydroxy group or a C_1-C_4 alkoxy group;
A_1 is Arg, Gly or 5-oxo-Pro (pGlu);
A_2 is Pro or ß- Ala;
A_3 is Lys or Asp;
A_4 is Gln, Asn or 5-oxo-Pro;
A_5 is Gln, Lys, Arg, N-acylated Arg or 5-oxo-Pro;
or wherein A_5 together with A_3 forms a cystine moiety;
A_6 is Phe or Tyr;
A_7 is Gly, Sar or Pro;
A_8 is Leu or Pro; and
s is 0, 1 or 2;
or a Tyr^0 derivative thereof.

Suitable examples of such a compound of the above general formula I are:

(1) H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (substance P).
(2) H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,
(3) H- -Ala-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,
(4) H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Tyr-Gly-Leu-Met-NH_2 and
(5) H-Arg-Pro-Cys-Pro-Gln-Cys-Phe-Tyr-Pro-Leu-Met-NH_2.

and their Tyr^0 derivatives.

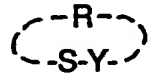
The invention also relates to the use of labelled small peptides as defined above, which are composed of amino acids, at least one of which has the d-configuration. The labelled peptides to be used according to the invention may also comprise so-called pseudo peptide bonds, viz. -CH_2-NH- bonds, in addition to the natural amide bonds, viz. -CO-NH- bonds.

The desired isotope as defined above should be firmly attached to the small peptide molecule to reduce detachment of this label after administration to

the living being. As will be clear from the above, the proper choice of the linker is an essential aspect of the present invention. The small peptid can be labelled with the desired halogen radioisotope directly or indirectly, viz. via a tyrosyl group. Direct labelling may be carried out, for example, by introducing a halogen atom or radioactive halogen atom into an activated aromatic group (e.g. tyrosyl or imidazolyl) present in the peptide molecule in a manner known per se, if desired followed by exchange with I-123, I-131, Br-75 or Br-76, e.g. by the method as described in European Patent 165630. In general, however, labelling via said linker is preferred, said linker being capable of reacting with an amino group, preferably a terminal amino group, of said peptide, and having a functional group for binding said isotope. By using said linker, the desired isotope can generally better be introduced into the peptide molecule. It is of advantage to attach the linker to a terminal amino group of the peptide molecule, in order to maintain the biological properties of this peptide as much as possible. It has been found that a tyrosyl linking group is very suitable for producing a radioactive-halogen-labelled peptide, which combines a selective affinity to NK1 receptors with a slow liver metabolic clearance. A tyrosyl moiety can be introduced into the amino acid chain, preferably in the 0-position, during the peptide synthesis; alternatively, the tyrosyl group can be introduced by a separate reaction of the peptide with tyrosine or a functional derivative thereof. The derivatized peptide, thus obtained, is substituted by the desired halogen radioisotope by an appropriate reaction. In this manner the peptide can be labelled with the desired radioactive halogen isotope without affecting its biological properties. The radiohalogenating reaction is preferably performed by reacting the peptide with a solution of an alkali metal radionuclide selected from $^{123}I^-$, $^{131}I^-$, $^{75}Br^-$ and $^{76}Br^-$ under the influence of a halide-oxidizing agent, such as chloramine T or iodogen. Alternatively, the above substitution reaction can be carried out with non-radioactive halogen, after which halo-exchange with radioactive halogen is performed, e.g. as described in European patent 165630.

A suitable linker for attaching a metal isotope to the small peptide is provided with a chelating group. Such isotopes are selected from the group consisting of Tc-99m, Pb-203, Ga-67, Ga-68, As-72, In-111, In-113m, Ru-97, Cu-62, Cu-64, Fe-52, Mn-52m, Cr-51, Na-23, Gd-157, Mn-55, Dy-162, Cr-52, Fe-56, Re-186, Re-188, As-77, Y-90, Cu-67, Er-169, Sn-121, Te-127, Pr-142, Pr-143, Au-198, Pd-109 and Dy-165. Various coupling agents for attaching metal isotopes to proteins are described in literature, such as compounds which after

coupling with the protein can complex the metal isotope by an N_2S_2-, N_3S- or N_4-tetradentate ring structure, amino-containing compounds such as the maleimide derivatives disclosed in European patent application 178125, peptide-derivatives, and compounds comprising chelating groups such as isocyanate, formyl, diazonium, isothiocyanate, alkoxycarbimidoyl groups and the like. Suitable linkers are derived from N-containing di- or polyacetic acids or their derivatives, such as ethylene diaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), ethylene glycol-0,0'-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), N,N'-bis(hydroxybenzyl)ethelenediamine-N,N'-diacetic acid (HBED), triethylenetetramine hexaacetic acid (TTHA), substituted EDTA or -DTPA, 1,4,7,10-tetra-azacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA) or 1,4,8,11-tetraazacyclotetradecane-N,N',N",N"'-tetraacetic acid (TETA). For modifying the small peptide in question, however, a linker is preferred which is derived from the coupling agents described in PCT application WO 89/07456. These coupling agents are generally be represented by the formula



(II)

wherein

R is a branched or non-branched, optionally substituted hydrocarbyl radical, which may be interrupted by one or more hetero-atoms selected from N, O and S and/or by one or more NH groups, and

Y is a group which is capable of reacting with an amino group of the peptide and which is preferably selected from the group consisting of carbonyl, carbimidoyl, N-(C_1-C_6)alkylcarbimidoyl, N-hydroxycarbimidoyl and N-(C_1-C_6)alkoxycarbimidoyl. Examples of coupling agents described therein are unsubstituted or substituted 2-iminothiolanes and 2-iminothiacyclohexanes.

The modification of the peptide in question, i.e. the reaction with the coupling agent, results in a peptide conjugate. This reaction can generally be carried out in a simple manner. In the subsequent complex-forming reaction, the metal isotope is presented to the peptide conjugate in the form of a salt or chelate. In the latter case relatively weak chelators are used, e.g. a phosphonate or polyphosphonate, an oxinate, a carboxylate, a hydroxycarboxylate, an aminocarboxylate or an enolate. Then the desired complex is formed by ligand exchange. The complex forming reactions can generally be carried out in a simple manner and under conditions which spare the peptide.

The invention further relates to a pharmaceutical composition to be used for the method as defined above, which composition comprises in addition to a pharmaceutically acceptable carrier and, if desired, at least one pharmaceutically acceptable adjuvant, as the active substance a small peptide having a selective affinity to neurokinine 1 receptors, said peptide being labelled with a detectable isotope as defined above. Such a composition is intended for diagnostic application, or, if labelled with a suitable isotope as indicated hereinbefore, for therapeutic application. If desired, the composition so obtained can be brought into a form more suitable for intravenous or subcutaneous application, e.g. by adding a pharmaceutically acceptable liquid carrier material. For intravenous or subcutaneous application the solution should of course be in a sterile condition.

The invention also relates to a labelled small peptide to be used as an active ingredient in the composition as described above, said peptide having a selective affinity to neurokinine 1 receptors and being labelled with an isotope as defined hereinbefore.

In case a radioactive labelled peptide is used as a diagnostic agent, it is frequently impossible to put the ready-for-use composition at the disposal of the user, in connection with the often poor shelf life of the radiolabelled compound and/or the short half-life of the radionuclide used. In such cases the user will carry out the labelling reaction with the radionuclide in the clinical hospital or laboratory. For this purpose the various reaction ingredients are then offered to the user in the form of a so-called "kit". It will be obvious that the manipulations necessary to perform the desired reaction should be as simple as possible to enable the user to prepare from the kit the radioactive labelled composition by using the facilities that are at his disposal. Therefore the invention also relates to a kit for preparing a radiopharmaceutical composition.

Such a kit according to the present invention may comprise (i) a small peptide having a selective affinity to neurokinine 1 receptors and optionally provided with a linking group as defined above, to which substance, if desired, an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (ii) a solution of a compound of a suitable radionuclide, and (iii) instructions for use with a prescription for reacting the ingredients present in the kit.

Suitable radionuclides for the above kit are: Pb-203, Ga-67, Ga-68, As-72, In-111, In-113m, Ru-97, Tc-99m, Re-186, Re-188, Cu-62, Cu-64, Fe-52, Mn-52m, Cr-51, I-123, I-131, Br-75, Br-76, As-77, Y-90, Cu-67, Er-169, Sn-121, Te-

127, Pr-142, Pr-143, Au-198, Pd-109 and Dy-165. If in such a kit the radionuclide is a radioactive halogen selected from I-123, I-131, Br-75 and Br-76, preferably an alkali metal halogenide, as generally known in the art, is used as an ingredient of the kit, if desired, accompanied by a halide oxidation agent, such as chloramine T or iodogen^{R}. Preferably the small peptide to be used as an ingredient of the above kit has been modified by a reaction with a coupling agent as defined hereinbefore. The resulting peptide conjugate provides a facility for firmly attaching the radionuclide in a simple manner. Suitable coupling agents for modifying the peptide are described in detail hereinbefore. N-containing di- or polyacetic acids or their derivatives, such as the compounds mentioned before, have proved to be pre-eminently suitable for attaching various metal radionuclides, such as In-111 and In-113m, to the peptide molecules. The kit to be supplied to the user may also comprise the ingredient(s) defined sub (i) above, together with instructions for use, whereas the solution of a compound of the radionuclide, defined sub (ii) above, which solution has a limited shelf life, may be put to the disposal of the user separately.

In case the kit serves to prepare a radiopharmaceutical composition labelled with Tc-99m, Re-186 or Re-188, such a kit according to the present invention may comprise, in addition to the ingredient(s) defined sub (i) above, (ii) a reducing agent and, if desired, a chelator, and (iii) instructions for use with a prescription for reacting the ingredients of the kit with Tc-99m in the form of a pertechnetate solution, or with Re-186 or Re-188 in the form of a perrhenate solution. If desired, the ingredients of the kit may be combined, provided they are compatible. The kit should comprise a reducing agent to reduce the pertechnetate or perrhenate, for example, a dithionite, a metallic reducing agent or a complex-stabilizing reducing agent, e.g. $SnCl_2$, Sn(II)-tartrate, Sn(II)-phosphonate or -pyrophosphate, or Sn(II)-glucoheptonate. The pertechnetate or perrhenate solution can simply be obtained by the user from a suitable generator.

In a preferred embodiment the kit according to the present invention comprises, a modified peptide or a peptide conjugate, obtained by modifying the peptide as defined hereinbefore by a treatment with a coupling agent. Suitable coupling agents have been described hereinbefore. The use of a compound of the general formula

R

S - Y

wherein the symbols have the meanings given hereinb fore, as a coupling agent is to be preferred.

When the radionuclide is present in the kit itself, the complex forming reaction with the peptide conjugate can simply be produced by combining the components in a neutral medium and causing them to react. For that purpose the radionuclide may be presented to the peptide conjugate in the form of a chelate bonded to a comparatively weak chelator, as described hereinbefore.

When the kit comprises a peptide conjugate as defined hereinbefore and is intended for the preparation of a radiopharmaceutical composition, labelled with Tc-99m, Re-186 or Re-188, the radionuclide will preferably be added separately in the form of a pertechnetate or perrhenate solution. In that case the kit will comprise a suitable reducing agent and, if desired, a chelator, the former to reduce the pertechnetate or the perrhenate. As a reducing agent may be used, for example, a dithionite or a metallic reducing agent. The ingredients may optionally be combined, provided they are compatible. Such a monocomponent kit, in which the combined ingredients are preferably lyophilized, is excellently suitable for being reacted, by the user, with the radionuclide solution. As a reducing agent for the above-mentioned kits is preferably used a metallic reducing agent, for example, Sn(II), Fe(II), Cu(I), Ti(III) or Sb(III); Sn(II) is excellently suitable. The peptide constituent of the above-mentioned kits, i.e. preferably the peptide conjugate, may be supplied as a solution, for example, in the form of a physiological saline solution, or in some buffer solution, but is preferably present in a dry condition, for example, in the lyophilized condition. When used as a component for an injection liquid it should be sterile, in which, when the constituent is in the dry state, the user should preferably use a sterile physiological saline solution as a solvent. If desired, the above-mentioned constituent may be stabilized in the conventional manner with suitable stabilizers, for example, ascorbic acid, gentisic acid or salts of these acids, or it may comprise other auxiliary agents, for example, fillers, such as glucose, lactose, mannitol, and the like.

The invention will now be described in greater detail with reference to the ensuing specific Examples.

EXAMPLE I

Synthesis of labelled peptides

Tyr^0-substance P is synthetized according to the solid-phase Merrifield method (J. Am. Chem. Soc. 85, 1963, 2149), using the so-called Fmoc-strategy (Int. J. Pept. Protein Res. 35, 1990, 161-214). This means that Fmoc-protected amino acids are successively coupled, each time followed by cleavage of the protecting Fmoc-group in basic medium.

DTPA-substance P is prepared by the method described by Hnatowich (US Patent 4,479,930) using lysine-protected substance P and DTPA-dianhydride. In a corresponding manner DTPA-Tyr^0-substance P is prepared.

Tyr^0-substance P is labelled with I-123 by dissolving this compound in phosphate buffer and adding an equimolar quantity of ^{123}I-sodium iodide to this solution in the presence of chloramine T. The labelling of the DTPA-modified peptides with In-111 is performed by dissolving said peptides in 0.01M acetic acid and mixing these solutions with ^{111}In-$InCl_3$ solution in 0.5M aqueous sodium acetate at room temperature. Then HEPES buffer is added for neutralizing purposes.

EXAMPLE II

Binding studies

Binding studies in vitro are carried out in a standardized system by studying the displacement of ^{125}I-Bolton Hunter substance P {[^{125}I]BH-SP} by the above-synthetized labelled peptides. Figure 1 shows that [^{125}I]BH-SP specifically binds to membranes of the cortex of rat brains and to human glioma membranes, but not to human meningeoma membranes.

The displacement of [^{125}I]BH-SP by iodinated Tyr^0-substance P is demonstrated by Figure 2; the measured IC_{50} value (nM) is 0.4 (cortex) and 0.7 (glioma). The displacement by In-complexed DTPA-Tyr^0-substance P is demonstrated by Figure 3; the IC_{50} value (nM) is 0.9 (cortex) and 3.2(glioma).

The results show that the labelled peptides of the invention bind specifically to the SP-receptors, present on the tissue membranes, because they are able to displace SP from these receptors.

EXAMPLE III

Liver perfusion experiments with labelled substance P

A liver perfusion model is used as described by Docter et al. in Endocrinology 1990, 126, 451-459. It is a generally accepted fact in this art, that handling of bio-active substances by the isolated perfused rat liver according to this model has a very good predictable value for the appearance of metabolites of the used substance in the circulation of human beings. In short, in this model isolated livers are perfused in a recirculating system with Krebs-Ringer medium supplemented with 1% bovine serum albumin and 10 mM glucose. After 0.5 hr preperfusion, experiments are started by addition of the labelled substance P (5 μCi) to the medium. At regular time points medium samples are taken. Medium samples are analyzed by column chromatography, using Seppak(R) C-18 columns.

The average results are presented in Figures 4-6, showing the disappearance of the total radioactivity ("tot. act.") and of the peptide-bound radioactivity ("protein") with concomitant appearance of non-peptide bound radioactivity in the circulating medium as a degradation product.

Figures 7-9 show the excretion of the tested labelled peptides into the bile.

In Figures 4 and 7 the results are presented of [^{125}I]BH-SP, showing that after 60 minutes, approximately 50% of the total radioactivity has disappeared, apparently to a considerable extent via liver metabolic clearance: approximately 25% of radioactivity is excreted into the bile. The differences with the tested labelled peptides of the invention are striking. The results of [^{123}I]Tyr^0-SP are presented in Figures 5 and 8, showing that after 60 minutes only about 15% of the total radioactivity has disappeared; the radioactivity excreted into the bile is only about 0.8%, rising to only about 1.2% after 120 minutes. Even more favourable results are obtained with [^{111}In]DTPA-Tyr^0-SP: Figures 6 and 9; only a negligible excretion of radioactivity into the bile can be observed after 120 minutes.

From the above results can be concluded that, as opposed to [^{123}I]BH-SP, the labelled peptides according to the invention are promising substances for scintigraphic imaging purposes with regard to handling by the liver. It can be concluded, that the metabolic clearance of these substances by the liver is negligible, resulting in a low background activity.

EXAMPLE IV

In vivo experiments

A pilot experiment with [^{111}In]DTPA-Tyr^0-SP is carried out in rats wherein granulomas are invoked. It is well-known that granulomas contain SP-receptors. The radiolabelled peptide is injected in a quantity corresponding to 500 µCi. A granuloma evoked in a leg of the rats can easily be visualized by scanning 2.5 hours after injection. The rats are sacrificed at four hours or 24 hours after injection, and various organs, including the granulomas, are excised and weighed. The measured radioactivity, corrected for the weight of the organs, is presented in Table 1 below. In Table 1, the radioactivity is expressed in ratios compared to the radioactivity in the blood, arbitrarily fixed at 1.0: relative radioactivity.

From the results it can be concluded, that a considerable accumulation of radioactivity, sufficiently for scanning purposes, takes place in the granulomas, and that the liver metabolic clearance is negligible in comparison with the renal clearance.

TABLE 1

Relative Radioactivity

	4 h a.i.	24 h a.i.	
ORGAN	Rat 1	Rat 2	Rat 3
granuloma	4.6	4.1	3.8
kidneys	195.2	295.8	309.6
liver	2.7	8.0	3.7
blood	1.0	1.0	1.0

CLAIMS:

1. A method for detecting and localizing tissues having neurokinine 1 receptors in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising, in a quantity sufficient for external imaging, a small peptide having a selective affinity to neurokinine 1 receptors, wherein said peptide is labelled with (a) a detectable metal isotope selected from the group consisting of Tc-99m, Pb-203, Ga-67, Ga-68, As-72, In-111, In-113m, Ru-97, Cu-62, Cu-64, Fe-52, Mn-52m, Cr-51, Na-23, Gd-157, Mn-55, Dy-162, Cr-52 and Fe-56, said metal isotope being attached to said peptide via a suitable linker capable of reacting with an amino group of said peptide, and having a chelating group for chelating said metal isotope, or with (b) a detectable halogen radioisotope selected from I-123, I-131, Br-75 and Br-76, said halogen radioisotope being attached to said peptide directly or via a linking tyrosyl group; and thereupon (ii) subjecting said being to external imaging to determine the targeted sites in the body of said being in relation to the background activity, in order to allow detection and localization of said tissues in said body.

2. A method for the therapeutic treatment of tumors having on their surface neurokinine 1 receptors in the body of a warm-blooded living being, which comprises administering to said being a composition comprising, in a quantity effective for combating or controlling tumors, a small peptide having a selective affinity to neurokinine 1 receptors, said peptide being labelled with a metal isotope selected from the group consisting of Re-186, Re-188, As-77, Y-90, Cu-67, Er-169, Sn-121, Te-127, Pr-142, Pr-143, Au-198, Pd-109 and Dy-165, said metal isotope being attached to said peptide via a suitable linker capable of reacting with an amino group of said peptide, and having a chelating group for chelating said metal isotope.

3. A method as claimed in any one of the preceding claims, which comprises administering to said living being a composition comprising a labelled peptide, derived from a compound of the general formula

$$R_1\text{-}(A_1)_m\text{-}A_2)_n\text{-}A_3)_o\text{-}Pro)_p\text{-}A_4)_q\text{-}A_5\text{-}Phe\text{-}A_6\text{-}A_7\text{-}A_8\text{-}Met(O)_s\text{-}R_2 \quad (I)$$

wherein all of the symbols m, n, o, p and q are 1, or all but one of the symbols m, n, o, p and q are 1 and the remaining symbol is 0;

R_1 is a hydrogen atom or a C_1-C_4 alkylcarbonyl group.

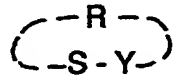
R_2 is an amino group, a hydroxy group or a C_1-C_4 alkoxy group;

A_1 is Arg, Gly or 5-oxo-Pro (pGlu);
A_2 is Pro or ß-Ala;
A_3 is Lys or Asp;
A_4 is Gln, Asn or 5-oxo-Pro;
A_5 is Gln, Lys, Arg, N-acylated Arg or 5-oxo-Pro;
or wherein A_5 together with A_3 forms a cystine moiety;
A_6 is Phe or Tyr;
A_7 is Gly, Sar or Pro;
A_8 is Leu or Pro; and
s is 0, 1 or 2;
or a Tyr^0 derivative thereof.

4. A method as claimed in claim 1 or 3, wherein said detectable halogen radioisotope is attached to a terminal amino group of said peptide via a tyrosyl linking group.

5. A method as claimed in claim 1, 2 or 3, wherein said linker, provided with a chelating group for chelating a metal isotope, is derived from ethylene diaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), ethylene glycol-0,0'-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), N,N'-bis(hydroxybenzyl)ethelenediamine-N,N'-diacetic acid (HBED), triethylenetetramine hexaacetic acid (TTHA), substituted EDTA or -DTPA, 1,4,7,10-tetra-azacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA) or 1,4,8,11-tetraazacyclotetradecane-N,N',N",N"'-tetraacetic acid (TETA).

6. A method as claimed in claim 1,2 or 3, wherein said linker is derived from a compound of the general formula



(II)

wherein
R is a branched or non-branched, optionally substituted hydrocarbyl radical, which may be interrupted by one or more hetero-atoms selected from N, O and S and/or by one or more NH groups, and
Y is a group which is capable of reacting with an amino group of the peptide and which is preferably selected from the group consisting of carbonyl, carbimidoyl,

N-(C_1-C_6)-alkylcarbimidoyl, N-hydroxycarbimidoyl and N-(C_1-C_6)alkoxycarbimidoyl.

7. A pharmaceutical composition to be used for the method as claimed in claim 1, comprising in addition to a pharmaceutically acceptable carrier and, if desired, at least one pharmaceutically acceptable adjuvant, as the active substance a small peptide having a selective affinity to neurokinine 1 receptors, said peptide being labelled with a detectable isotope as defined in claim 1.

8. A pharmaceutical composition to be used for the method as claimed in claim 2, comprising in addition to a pharmaceutically acceptable carrier and, if desired, at least one pharmaceutically acceptable adjuvant, as the active substance a small peptide having a selective affinity to neurokinine 1 receptors, said peptide being labelled with an isotope as defined in claim 2.

9. A composition as claimed in claim 7 or 8, comprising as the active substance a labelled small peptide derived from a compound of the general formula I, presented in claim 3, wherein the symbols have the meanings given in claim 3.

10. Use of a composition as claimed in any one of claims 7 and 9, for the manufacture of a diagnostic agent for detecting and localizing tissues having neurokinine 1 receptors in the body of a warm-blooded living being.

11. Use of a composition as claimed in any one of claims 8 and 9 for the manufacture of a therapeutic agent for treating tumors having on their surface neurokinine 1 receptors in the body of a warm-blooded living being.

12. A labelled small peptide to be used as an active ingredient in a composition as claimed in claim 7 or 8, said peptide having a selective affinity to neurokinine 1 receptors and being labelled with an isotope as defined in claim 1 or 2, respectively.

13. A labelled small peptide as claimed in claim 12; said peptide being derived from a compound of th general formula I, presented in claim 3, wherein the symbols have the meanings given in claim 3.

14. A kit for preparing a radiopharmaceutical composition, comprising (i) a small peptide having a selective affinity to neurokinine 1 receptors and optionally provided with a linking group as defined in claim 1, to which substance, if desired an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (ii) a solution of a compound of a radionuclide selected from the group consisting of Pb-203, Ga-67, Ga-68, As-72, In-111, In-113m, Tc-99m, Re-186, Re-188, Ru-97, Cu-62, Cu-64, Fe-52, Mn-52m, Cr-51, I-123, I-131, Br-75, Br-76, As-77, Y-90, Cu-67, Er-169, Sn-121, Te-127, Pr-142, Pr-143, Au-198, Pd-109 and Dy-165, and (iii) instructions for use with a prescription for reacting the ingredients present in the kit.

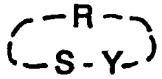
15. A kit for preparing a radiopharmaceutical composition, comprising (i) a small peptide having a selective affinity to neurokinine 1 receptors and optionally provided with a linking group as defined in claim 1, to which substance, if desired, an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (ii) a reducing agent and, if desired, a chelator, said ingredients (i) and (ii) optionally being combined, and (iii) instructions for use with a prescription for reacting the ingredients of the kit with Tc-99m in the form of a pertechnetate solution or with Re-186 or Re-188 in the form of a perrhenate solution.

16. A kit as claimed in claim 14 or 15, comprising as a small peptide a compound of the general formula I, presented in claim 3, wherein the symbols have the meanings given in claim 3.

17. A kit as claimed in any one of the claims 14-16, comprising a peptide conjugate, obtained by modifying a small peptide, as defined in claim 1 or 3, by a reaction with a linker having a functional group for binding or chelating a radionuclide as defined in claim 1.

18. A kit as claimed in claim 17, comprising a peptide conjugate obtained by modifying said small peptide by a reaction with a linker, said linker

being derived from ethylene diaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), ethylene glycol-0,0'-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), N,N'-bis(hydroxybenzyl)ethelenediamine-N,N'-diacetic acid (HBED), triethylenetetramine hexaacetic acid (TTHA), substituted EDTA or-DTPA, 1,4,7,10-tetra-azacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) or 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA), or from a compound of the general formula



(II)

wherein

R is a branched or non-branched, optionally substituted hydrocarbyl radical, which may be interrupted by one or more hetero-atoms selected from N, O and S and/or by one or more NH groups, and

Y is a group which is capable of reacting with an amino group of the peptide and which is preferably selected from the group consisting of carbonyl, carbimidoyl, N-(C_1-C_6)- alkylcarbimidoyl, N-hydroxycarbimidoyl and N-(C_1-C_6)alkoxycarbimidoyl.

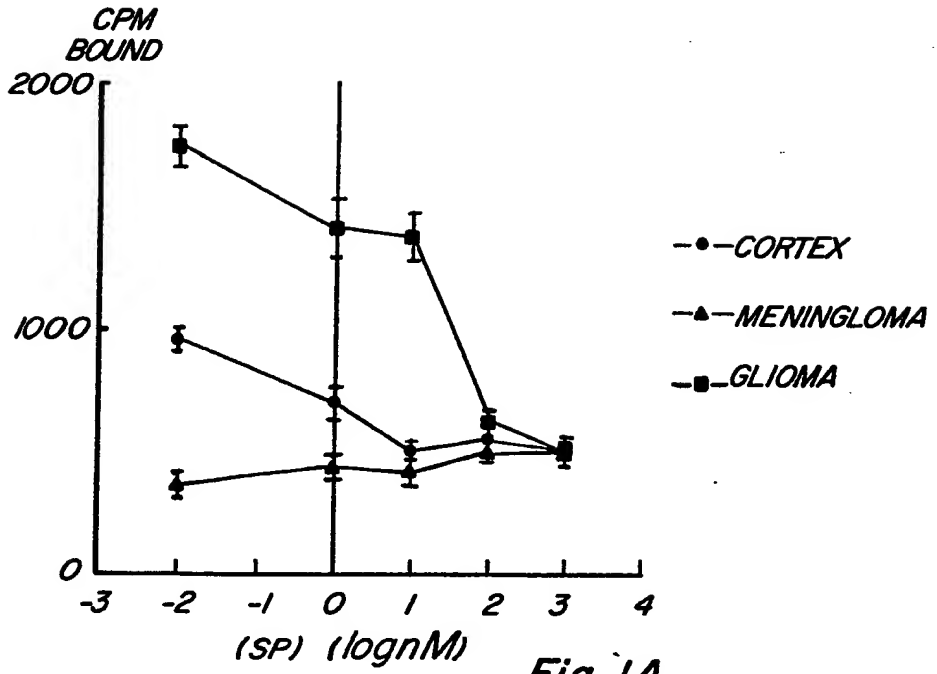

CPM
BOUND
2000
1000
0
-3
-2
-1
0
1
2
3
4
(SP) (lognM)
CORTEX
MENINGLOMA
GLIOMA

Fig. 1A

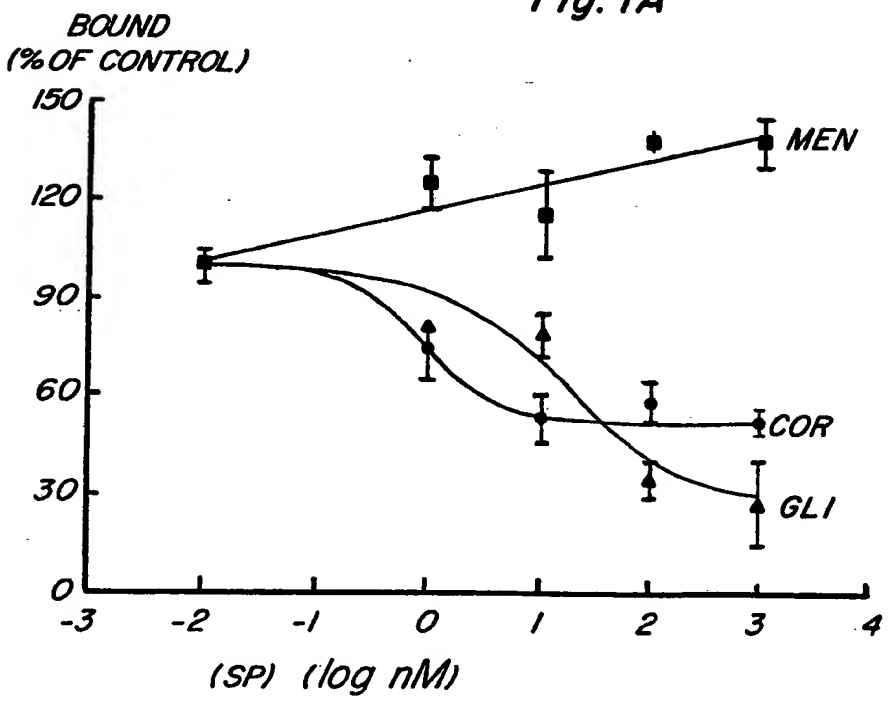

BOUND
(% OF CONTROL)
150
120
90
60
30
0
-3
-2
-1
0
1
2
3
4
(SP) (log nM)
MEN
COR
GLI

Fig. 1B

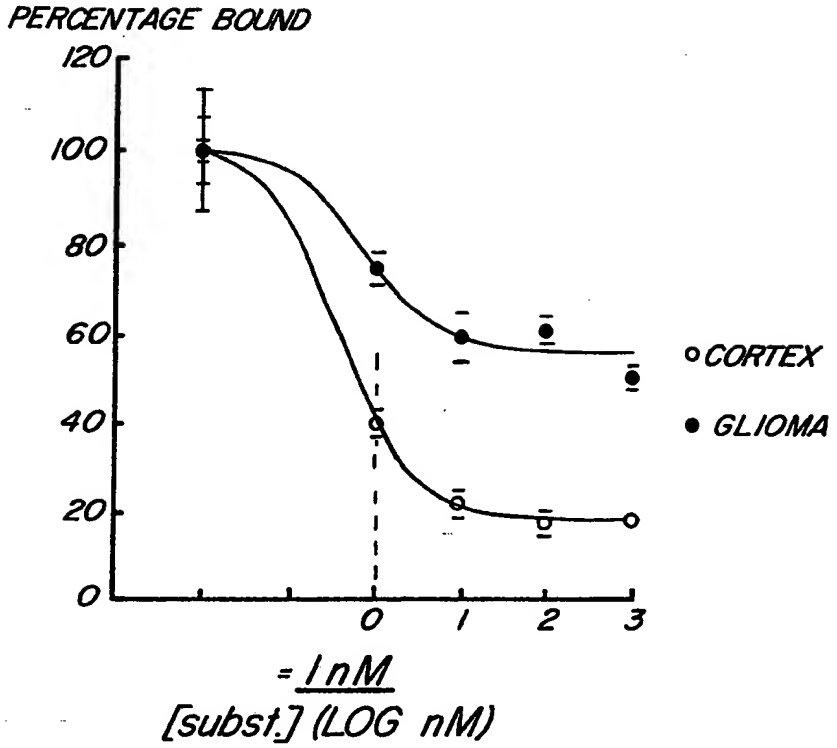

PERCENTAGE BOUND
120
100
80
60
40
20
0
0
1
2
3
CORTEX
GLIOMA
= 1nM
[subst.] (LOG nM)

Fig. 2

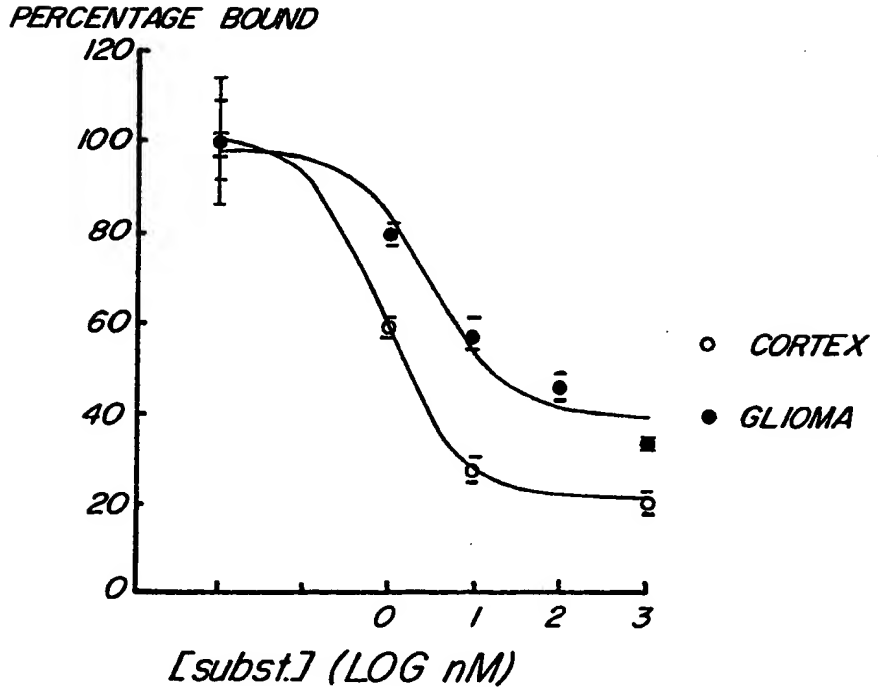

PERCENTAGE BOUND
120
100
80
60
40
20
0
0
1
2
3
CORTEX
GLIOMA
[subst.] (LOG nM)

Fig. 3

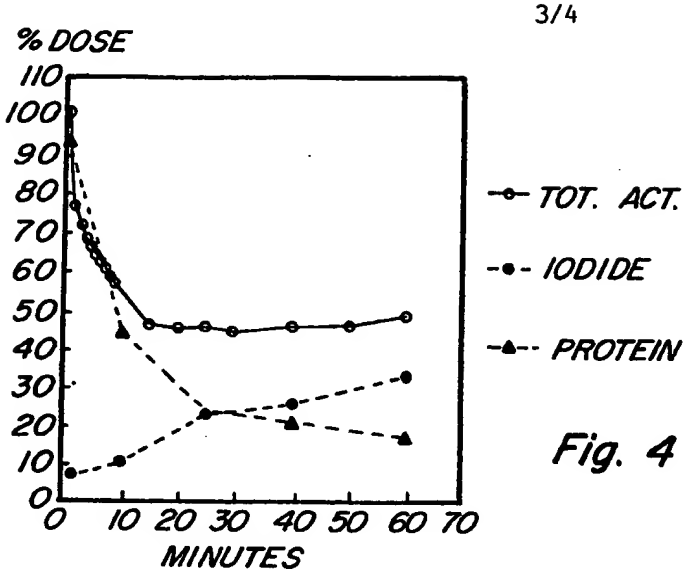


Fig. 4

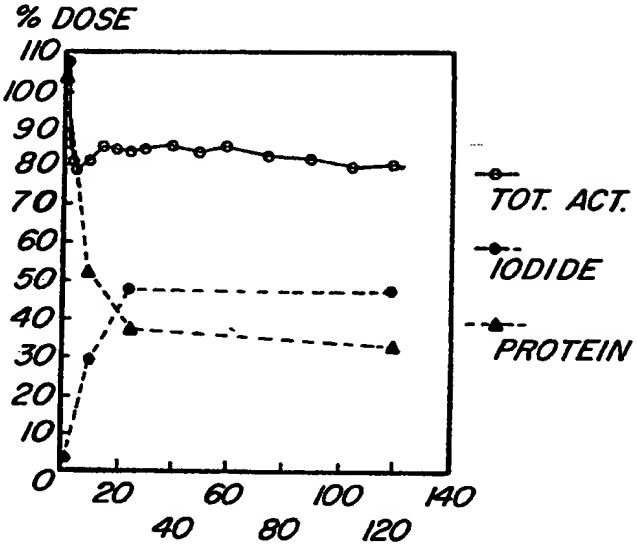


Fig. 5

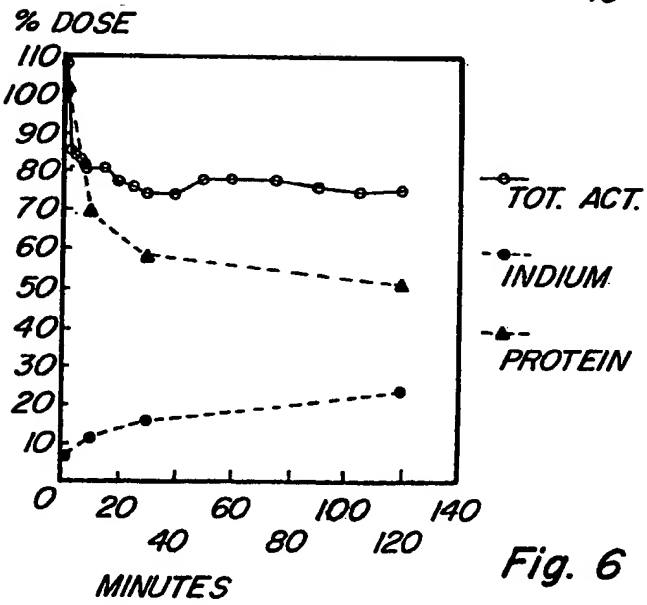


Fig. 6

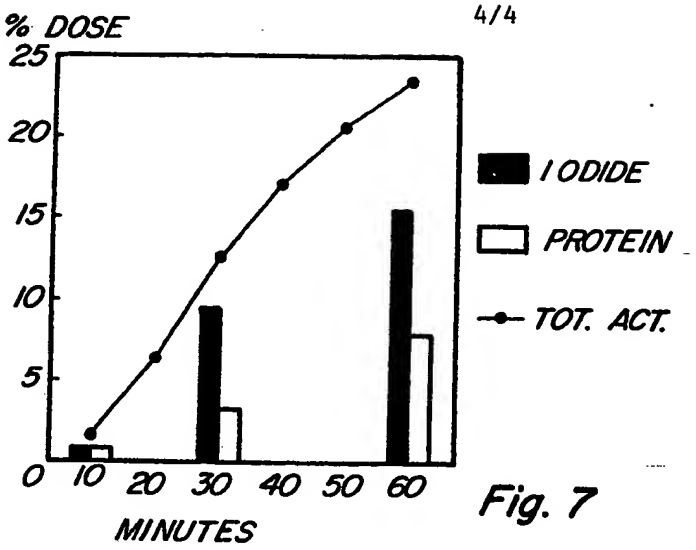


Fig. 7

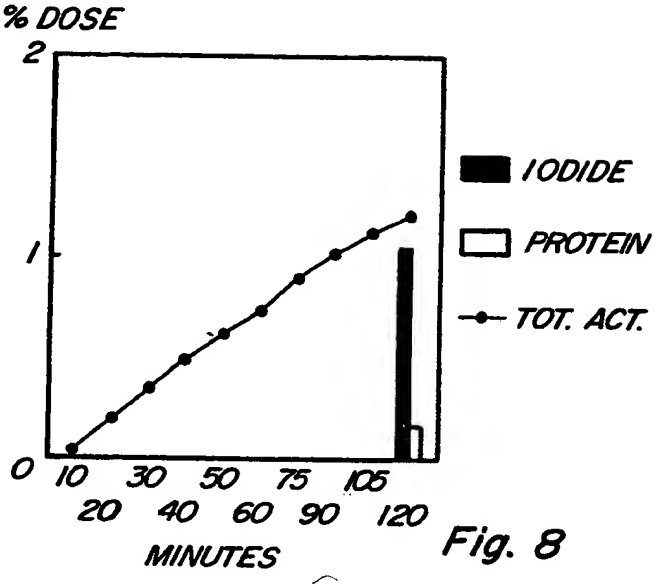


Fig. 8

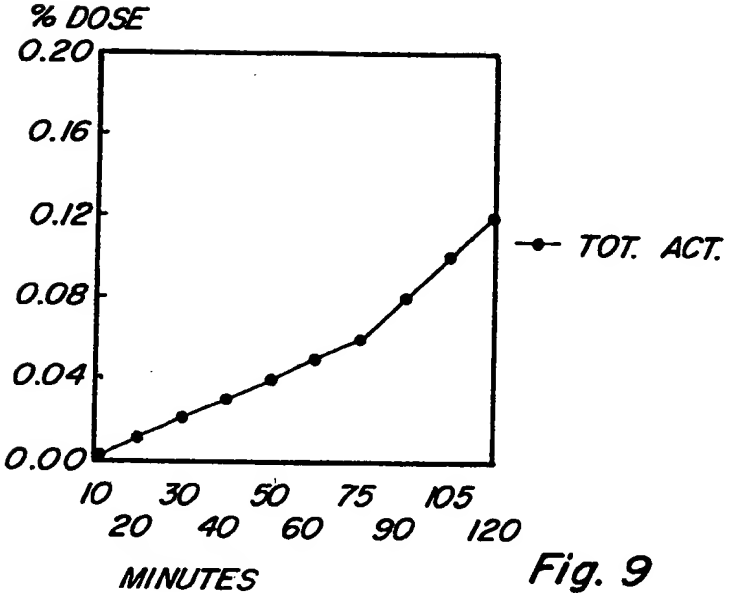


Fig. 9